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14. ABSTRACT

This year we have confirmed that hNPC delivering GDNF can protect dopamine neurons in a rat model of Parkinson's disease and have now shown an improvement in behavioral asymmetry induced by the lesion. We have completed a large monkey study using GDNF-hNPC and are in the process of analyzing those data. We have published regulated vector data described last year and have two other papers under review for publication. We were awarded a one year no cost extension with (new aims and goals) to this grant which is currently underway.

15. SUBJECT TERMS
Stem cells

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Introduction

This is the yearly update for our DOD award describing progress made for the fourth year - April 1, 2006 and April 1, 2007. The aims for this past year were to publish the data presented in last year's report and continue exploring the use of regulated vector systems. In light of current clinical trials using growth factors other than GDNF, we also explored the potential of insulin-like growth factor (IGF-1) and compared its effects to GDNF, which haven't been done using hNPC gene delivery in a Parkinson's model. Specifically, we feel we are making great strides in understanding if hNPC delivering GDNF or IGF-1 can "**prevent neurotoxic cell death and how this relates to dopamine storage in the brain using micro-PET (Task 2, part C)."** Although our progress has been delayed using the regulated vector system, we are now clearly showing that hNPC delivering GDNF can prevent dopamine neuron loss as well as reverse amphetamine-induced behavior in a rat model of Parkinson's disease. Interestingly, IGF-1 appears to have an equivalent beneficial effect to GDNF in this model. Finally, we have concluded the large monkey MPTP study and are currently analyzing the brain sections for anatomical effects of GDNF release, but there is an indication that hNPC expressing GDNF can reduce overall behavioral abnormalities associated with the MPTP lesion.

Body

Task 1. **To produce rat and monkey neural stem cells which secrete GDNF under an inducible promoter.**

a. Assess and optimize GDNF release from rat and monkey neurospheres using lentiviral vectors (Months 1-18).

Has been addressed in previous reports.

b. Establish DOX regulation profile on GDNF secreted from rat and monkey neurospheres infected with lentivirus (Months 6-24).

Completed last year.

c. Select and characterize lentiviral clones which express GDNF at high levels following differentiation (Months 18-36).

Discussed in last year's report.

d. Produce and optimize new vectors with combinations of GDNF/GFP (retrovirus/AAV) - assess which is most efficient at GDNF production *in vitro* and compare with lentivirus (Months 6 - 48).

As explained in last year's report, we have begun our collaboration with Rehogene (http://www.rheogene.com/) using their promoter/inducer technology to regulate GDNF expression in hNPC. We have collected preliminary data using transient transfection of the Rheogene plasmid containing GDNF in hNPC. After confirming that the inducer (RG115819) was not toxic to hNPC, we observed that GDNF was produced by transfected cells in the presence of the inducer (Fig 1). A lentivirus was then produced by our Swiss collaborators using the Rheogene plasmid (lenti-RG-GDNF). Experiments are currently underway to determine the infection efficiency of the virus in hNPC and then we will begin experiments to test GDNF regulation *in vitro* and *in vivo*.

Task 2. **To protect against toxic cell death in the brain by transplanting GDNF producing stem cells into rodent and primate models of PD.**

a. Perform pilot monkey transplant study with GFP/GDNF retroviral construct using micro-PET and post mortem data to establish survival and possible function of cells (Months 6-18).

This issue has been addressed in previous reports.

b. Assess optimal source and preparation (FACS, pre-differentiation) of rodent neural stem cells for grafting (Months 12-48).

As described in detail in last year's report, we have determined that the specific lesioned environment impacts the migration and survival properties of hNPC transplanted into the rat brain. These data were submitted for publication and are currently under review.

c. Assess whether rodent GDNF secreting stem cells prevent neurotoxic cell death and how this relates to dopamine storage in the brain using micro-PET (Months 18-36)

This year we have also reassessed the efficacy of GDNF secreting cells on the behavioral asymmetries that develop after a 6-OHDA striatal lesion in rats. In our previous work we had determined that GDNF expressing hNPC could prevent dopamine neuron death in the substantia nigra and reduce amphetamine-induced rotations in this rat model of PD by 6 weeks after transplantation (Behrstock et al., 2006). However, we also observed a spontaneous recovery in the lesion control rats by 10 weeks post-lesion suggesting that our lesion paradigm was not optimal (Behrstock et al., 2006). We have since improved our lesion paradigm using a 3 site injection model based on the paper by Kirik and colleagues (1998) and have now shown a consistent lesion over 12 weeks with no spontaneous recovery.

GDNF has long been established as the growth factor of choice for neuroprotective and neurorestorative therapies for dopamine neurons. Yet there is some evidence in the literature that insulin-like growth factor (IGF-1) also has neuroprotective benefits for dopamine neurons in rat models of Parkinson's disease (Guan et al., 2000; Krishnamurthi et al., 2004; Quesada and Micevych, 2004). Furthermore, IGF-1 has also shown positive effects in other neurodegenerative models including amyotrophic lateral sclerosis (ALS) and stroke (Kaspar et al., 2003; Cao et al., 2003; Guan et al., 2001).

We undertook an experiment to test *ex vivo* gene therapy using GDNF and IGF-1 in the 6-OHDA lesion model of PD. This experiment had two main aims: 1. To reevaluate the protective effect of GDNF on dopamine neurons and amphetamine-induced rotation using the optimized lesion paradigm, and 2. To directly compare GDNF to IGF-1 secreting hNPC on dopamine neuron survival and behavioral recovery. We also included 2 control groups consisting of uninfected hNPC and a sham transplant of dead cells to determine if the cells themselves or the surgical procedure have any beneficial effect in this model. Rats were lesioned with 6-OHDA and then transplanted with the appropriate cells 1 week later. Rats were then tested for amphetamine induced rotations every other week for 12 weeks after the transplant. We found that both GDNF and IGF-1 secreting hNPC provide similar behavioral recovery whereas neither uninfected hNPC nor a sham transplant had any behavioral recovery (Fig 2). Upon histological analysis of brain sections, both GDNF and IGF-1 hNPC groups had significantly more dopamine neurons remaining compared to either control group (Fig 3). Interestingly though, only the GDNF group had protection of the dopamine fibers in the striatum (Fig 4). When we assessed hNPC survival in the brain, hNPC secreting IGF-1 showed increased hNPC survival compared to the other groups, which was consistent with what we observed *in vitro* (Fig 5).

Taken together, these data suggest that although GDNF and IGF-1 provide similar behavioral recovery and protection of dopamine neurons, they appear to be exerting these benefits through different means. For example, GDNF hNPC protect the fibers in the striatum which may attribute to the behavioral recovery and dopamine survival. On the other hand, IGF-1 has increased hNPC survival which could be providing additional and/or longer periods of growth factor release leading to improved neuron survival and behavioral recovery. These data have been submitted for publication and are currently under review.

Finally, there is evidence in the literature that a combined treatment of GDNF and IGF-1 provide synergistic benefits in models of cerebellar degeneration (Bilak and Kuncl, 2001; Bilak et al., 2001; Tolbert and Clark, 2003), and we are currently testing this possibility in the PD model.

Last year we mentioned beginning a large primate experiment to test the efficacy of GDNF expressing hNPC to build off of our positive pilot data. 20 young cynomologous monkeys were treated with MPTP and were divided into 4 treatment groups for transplant 1 week post-MPTP: 1. media, 2. lentiGDNF, 3. hNPC, 4. hNPC-GDNF. The lentiGDNF group was used as a comparison control for the hNPC-GDNF group because of previous data showing substantial protection in this primate model (Kordower et al., 2000). Monkeys were tested for clinical rating scores prior to MPTP, prior to transplant, and every week following transplant for 3 months. All monkeys have been sacrificed and the brains are being processed. We have found that there was a modest improvement in average clinical rating in monkeys receiving either lentiGDNF or hNPC-GDNF. Interestingly, there was also a slight improvement in monkeys transplanted with uninfected hNPC. There was no recovery in monkeys receiving only media (Fig 6). Importantly, we found that the hNPC-GDNF cells did produce GDNF in the area around the transplant site (Fig 7). We are now in the process of analyzing the histological data to determine how well the transplanted cells survived and what if any impact the transplanted cells had on the dopamine neurons.

d. Prove regulated delivery can be achieved, and establish effects of switching GDNF on and off on dopamine storage in the brain using micro-PET (Months 24- 48).

As was described in detail in last year's report, the single vector tTrk promoter system driving GDNF expression in hNPC transplanted into the rodent brain did not produce GDNF to a sufficient level to be detected. These data were recently published (Capowski et al., 2007). Furthermore, we are in the process of investigating the Rheogene inducible vector system which will be used for micro-PET studies.

Key research accomplishments

This year we have published a paper describing the use of the regulatable tTrk vector system in hNPC *in vitro* and *in vivo*. We have also established that the lesion environment is crucial for both the migration and survival of transplanted cells, and these data are currently under review for publication. We have yet to perform PET scans in animals with transplants as the *in vivo* regulation has not yet been validated, but we are working toward completing those experiments with the new Rheogene vector system. We have shown the hNPC secreting GDNF can survive in the MPTP lesioned primate brain and provide a modest improvement in overall behavioral impairments. Finally, we have shown that hNPC secreting GDNF can provide behavioral recovery in a rat model of PD, and that hNPC secreting IGF-1 are as effective as GDNF.

Reportable Outcomes

1. AD Ebert, AJ Beres, AE Barber, and CN Svendsen. Human neural progenitor cells modified to release IGF-1 protect dopamine neurons and restore function in a rat model of Parkinson's disease. Submitted

2. S. Behrstock, AD Ebert, S Klein, M Schmitt, J Moore, and CN Svendsen. Lesion-induced increase in survival and migration of human neural progenitor cells releasing GDNF. Submitted

3. EE Capowski, BL Schneider, AD Ebert, CR Seehus, J Szulc, R Zufferey, P Aebischer, and CN Svendsen. Lentiviral vector-mediated genetic modification of human neural progenitor cells for ex vivo gene therapy. *J Neurosci Methods*. 163(2):338-49, 2007.

4. M Suzuki, J McHugh, C Tork, B Shelley, SM Klein, P Aebischer, and CN Svendsen. GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. PLoS ONE (in press), 2007.

5. S. Behrstock, A. Ebert, J. McHugh, S. Vosberg, J. Moore, B. Schneider, E. Capowski, D. Hei, J. Kordower, P. Aebischer, and C. N. Svendsen. Human neural progenitors deliver glial cell line-derived neurotrophic factor to parkinsonian rodents and aged primates. *Gene Ther.* 13 (5):379-388, 2006.

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8. A. D. Ebert and C. N. Svendsen. A new tool in the battle against Alzheimer's disease and aging: ex vivo gene therapy. *Rejuvenation.Res.* 8 (3):131-134, 2005.

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Papers were presented at the Society of Neuroscience meeting. Dr. Svendsen has given over 20 invited lectures over the past year presenting aspects of the current work both in the USA and abroad.

Conclusions

We feel that good progress has been made this year towards our original goals. We conclude that human neural progenitor cells modified to release GDNF remain a potential source of tissue for new cellular therapies for Parkinson's disease. By learning more about stem cell drug delivery it may be possible to explore other therapies for war injuries in the future.

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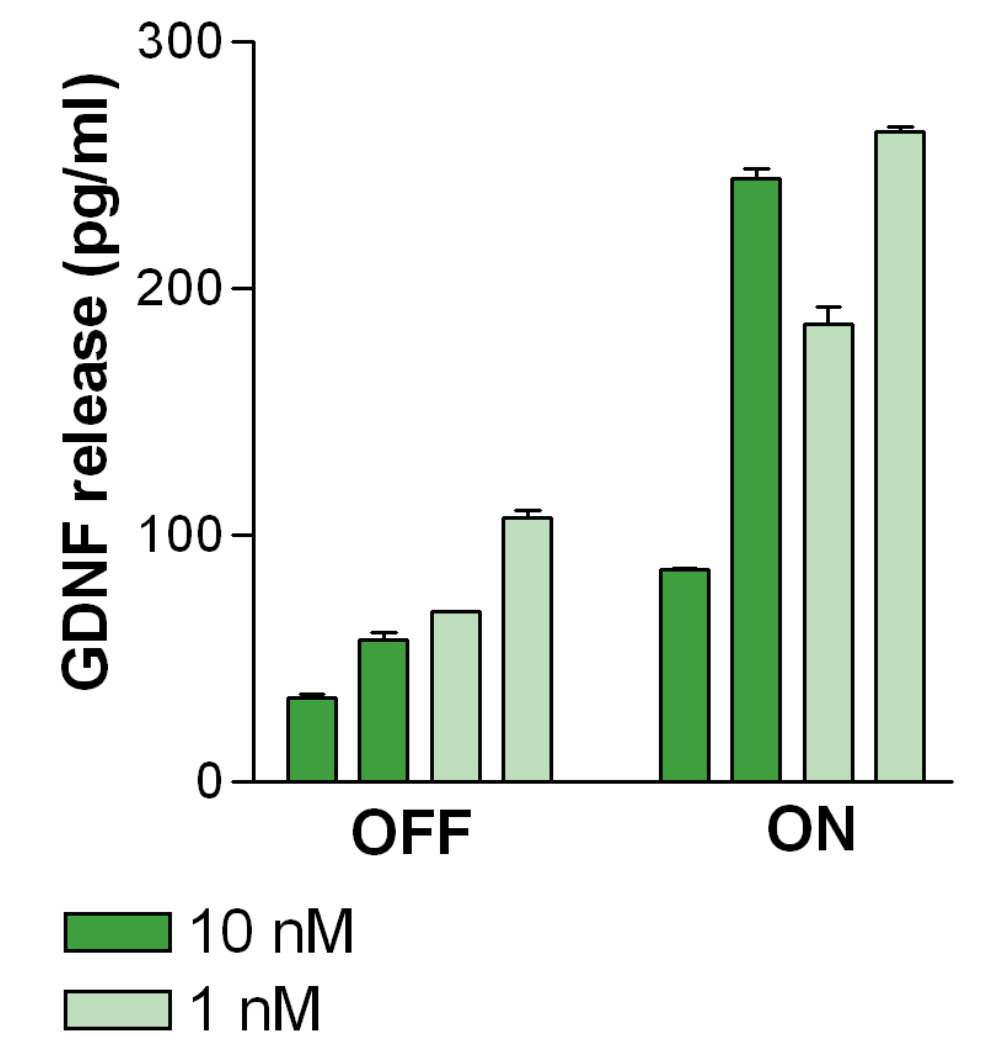
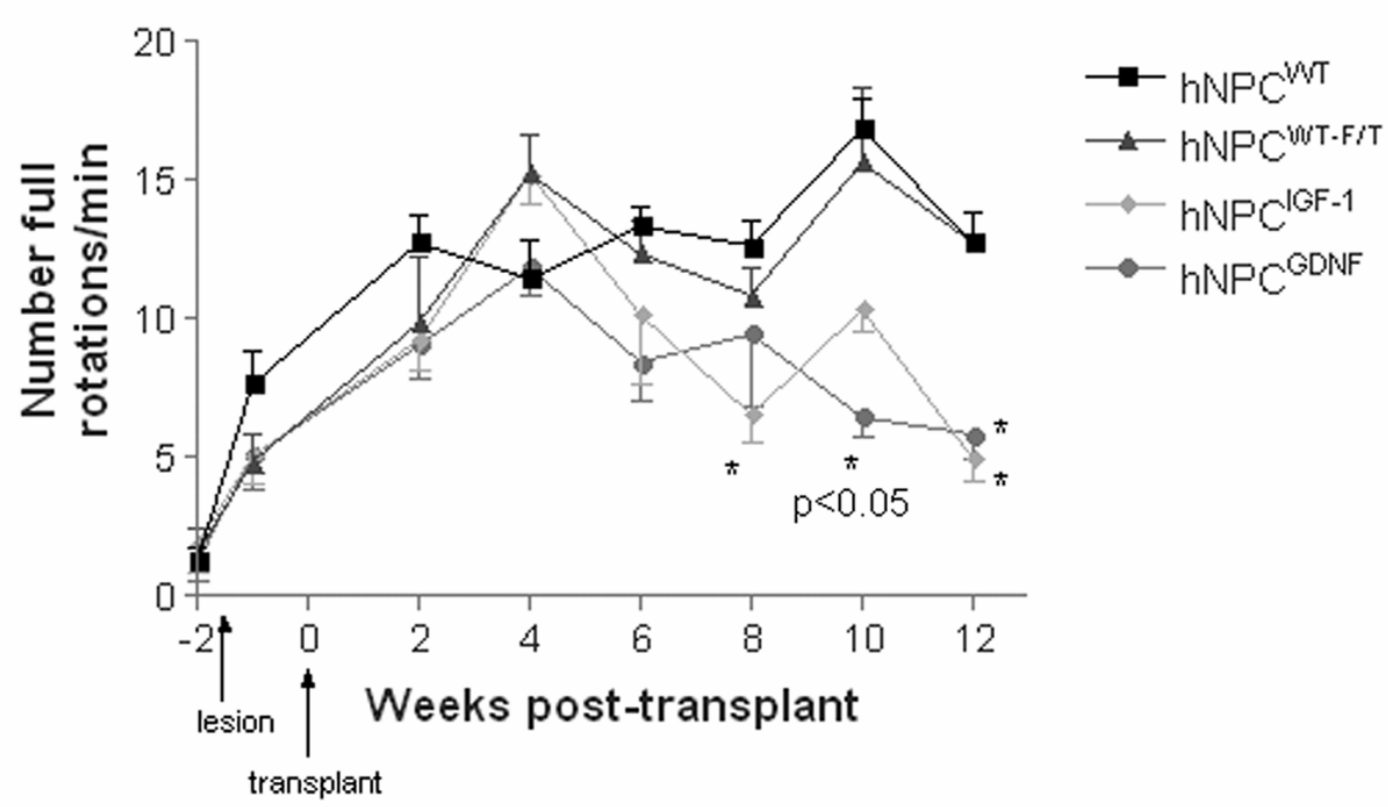
Tolbert, DL & Clark, BR (2003) Exp Neurol 183(1):205-219.

Figure 1



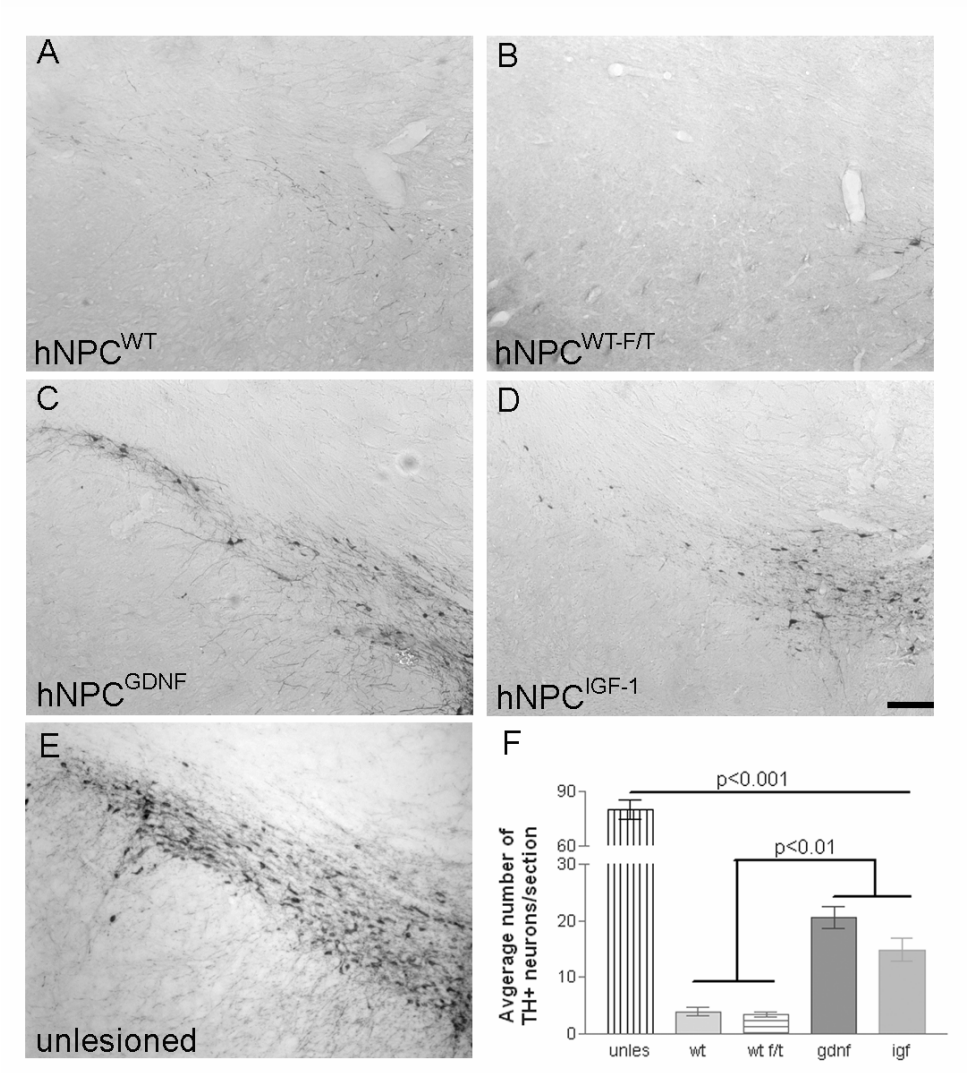
Transfection of Rheogene plasmid in hNPC. The presence of inducer increased the amount of GDNF secreted from transfected hNPC *in vitro*.

Figure 2



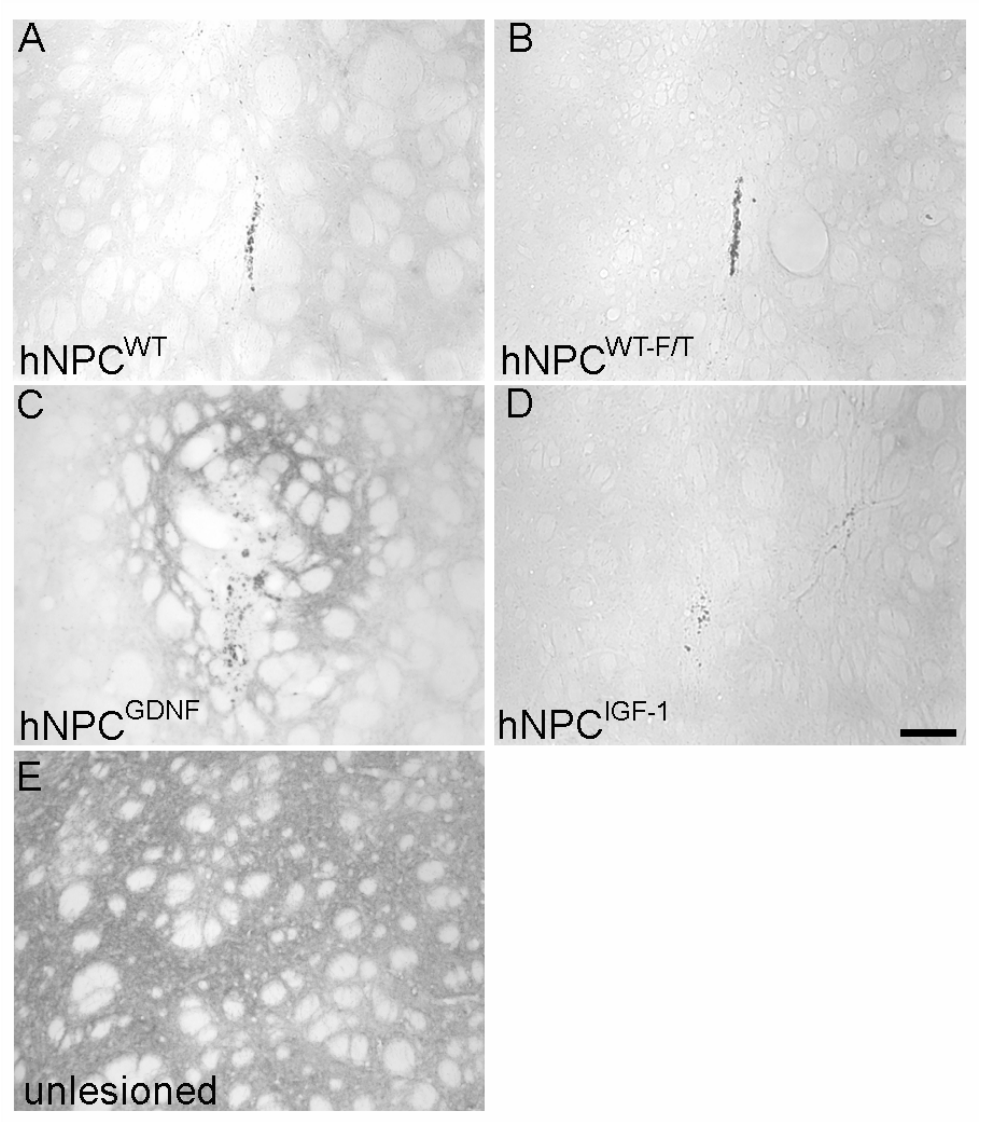
Transplantation of hNPC secreting either GDNF or IGF-1 reverses amphetamine induced rotational asymmetry.

Figure 3



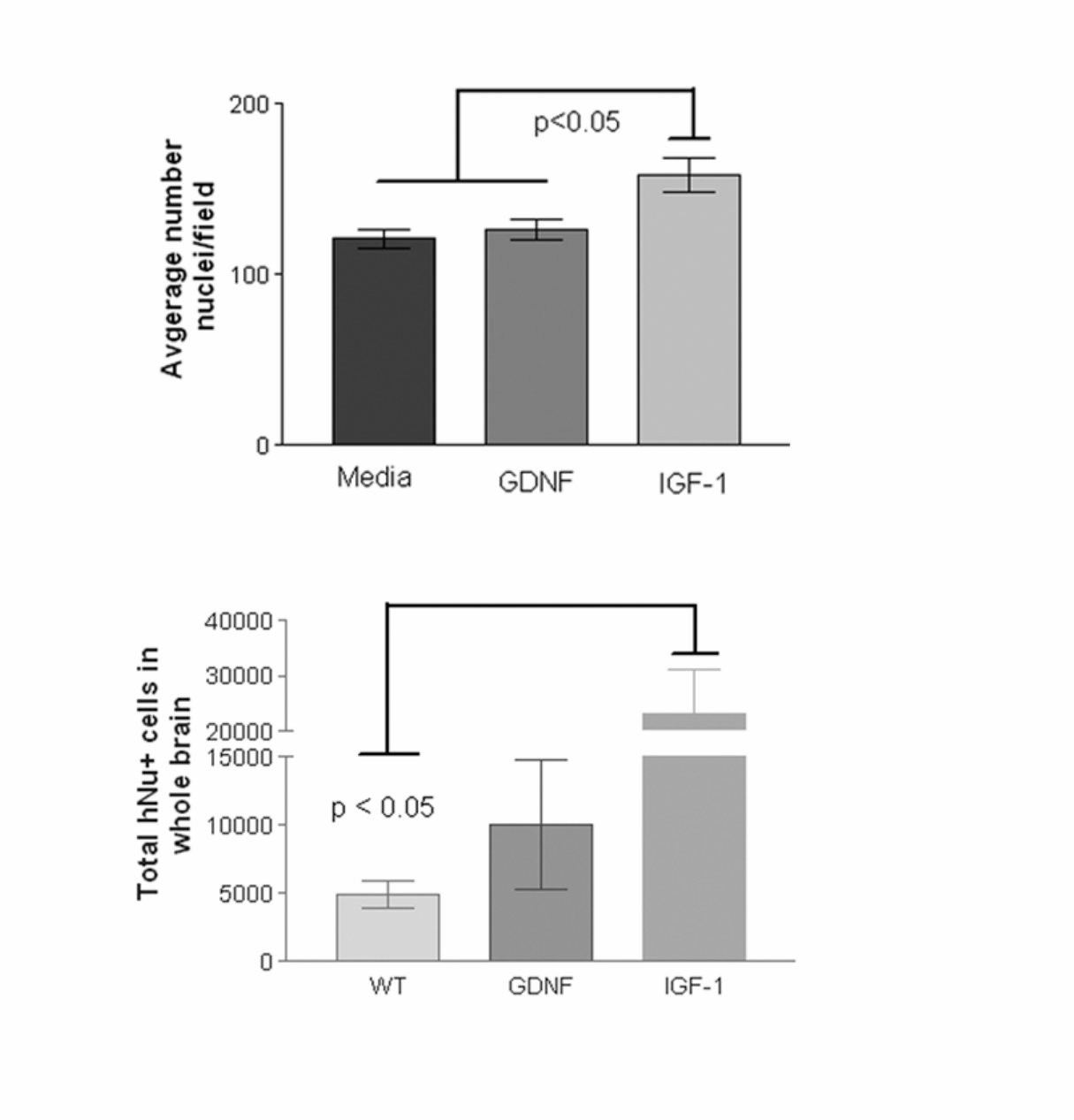
Both GDNF and IGF-1 secreting hNPC provide significant protection of dopamine neurons in the SN that are affected by the 6-OHDA lesion. Neither uninfected hNPC nor a sham transplant provided any neuronal protection.

Figure 4



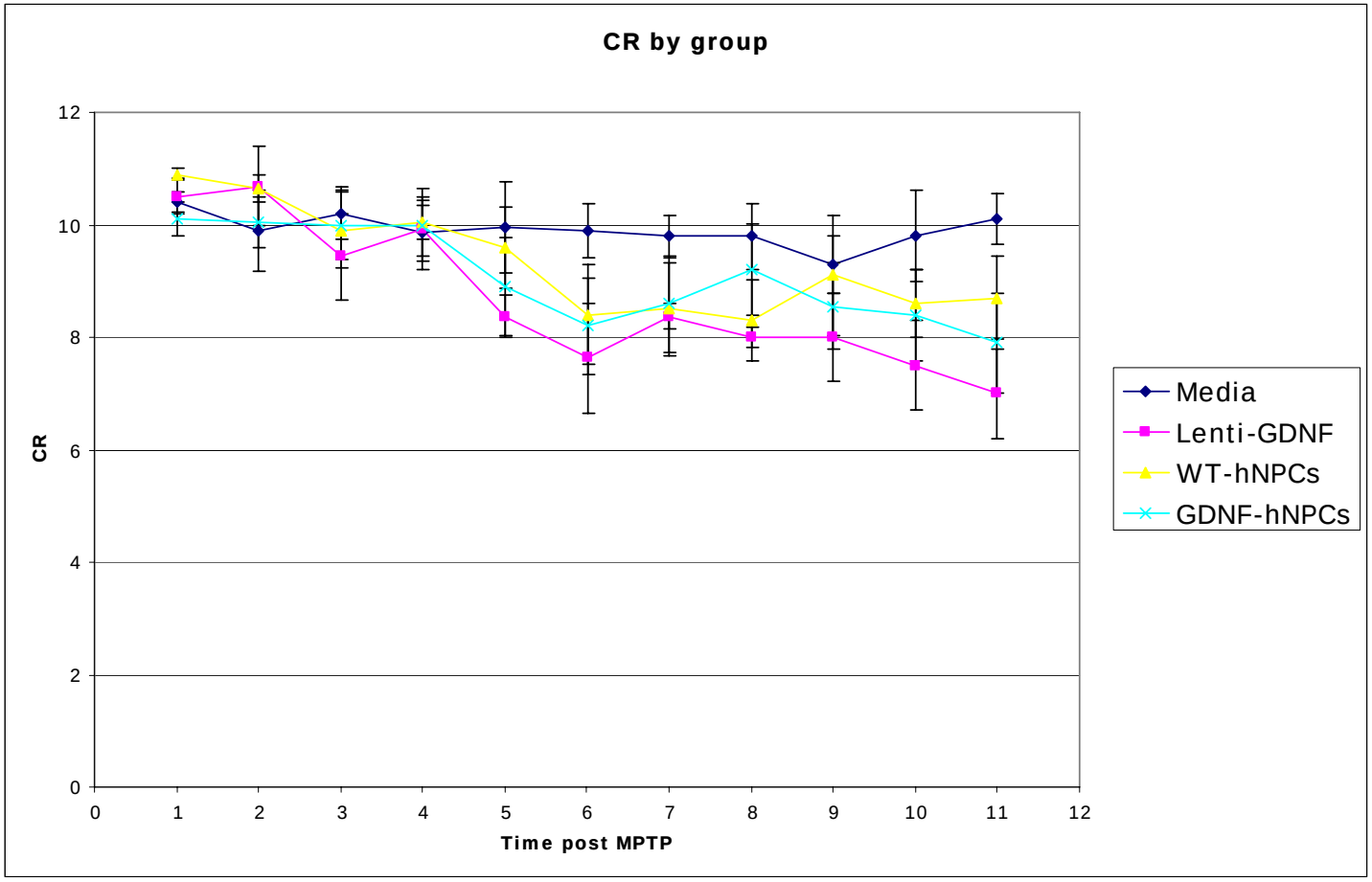
Only hNPC secreting GDNF protected or regenerated dopamine fibers in the striatum.

Figure 5



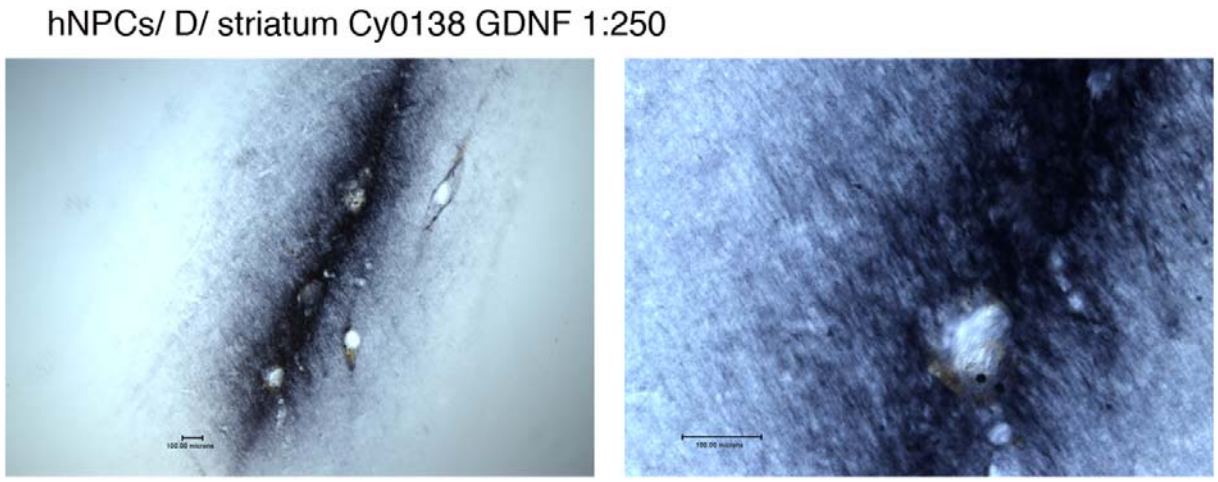
hNPC secreting IGF-1 had more cells survive in culture (top graph) and after transplantation into the brain (bottom graph).

Figure 6



LentiGDNF and hNPC secreting GDNF each provided some recovery in clinical rating scores in MPTP treated monkeys. Uninfected hNPC also showed a modest improvement in clinical rating scores. Data is currently being analyzed.

Figure 7



hNPC-GDNF cells continued to produce GDNF in the brain of young MPTP treated monkeys 3 months after the transplant. Data is currently being analyzed.